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Bioethanol production from pentose sugars: Current status and future prospects

Ramesh Chander Kuhad[a,*], Rishi Gupta[a], Yogender Pal Khasa[a], Ajay Singh[b], Y.-H. Percival Zhang[c]

[a] *Lignocellulose Biotechnology Laboratory, Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India*
[b] *Lystek International Incorporation, 107-279 Weber Street North, Waterloo, Ontario, Canada N2J3H8*
[c] *Biological Systems Engineering Department, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*

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ABSTRACT

The utilization of hemicellulose, the second most abundant polysaccharide, is must for the cost-efficient production of ethanol from second generation feedstocks. Xylan, the major hemicellulose in plant biomass yields mainly xylose as pentose sugars on hydrolysis. The progress in fermentation of pentose sugars has gone on slow pace as there are few microorganisms known, which are capable of pentose metabolism. The future perhaps lies in finding organisms that would ferment high density hydrolysates without purification. This obviously has to use the genetic and metabolic engineering routes. Either a direct or a sequential fermentation system needs to be worked out. This review provides an overview of the current pentose bioconversion processes and future prospects for bioethanol production.

Contents

* Corresponding author. Tel.: +91 11 24112062; fax: +91 11 24115270; mobile: +91 9871509870.
E-mail address: kuhad85@gmail.com (R.C. Kuhad).

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1. Introduction

Utilization of lignocellulosic biomass for ethanol production is one of the most promising alternatives for liquid fuel, which has near zero green house gas emission with great socio-economic benefits [1–4]. Lignocellulose is the most abundant, renewable organic material on the biosphere, but by virtue of its structural properties they are resistant to bioconversion [5–7]. For cost-effective production of bioethanol from lignocellulosic biomass, the high-efficiency utilization of both carbohydrate fractions, i.e., cellulose and hemicellulose is required.

Bioethanol production from lignocellulosic biomass involves several steps such as pretreatment, hydrolysis of complex carbohydrates, fermentation, and distillation for product recovery [8,9]. Various pretreatment methods have been explored to enhance the accessibility of lignocellulosic substrates. Among them, dilute acid pretreatment is the method of choice which has been studied widely [9–13]. However, it has drawback of non-selectivity and formation of fermentation inhibitory compounds such as formic acid, levulinic acid, furfural, 5-hydroxymethylfurfural and phenolics [9,14,15]. In order to remove these toxic compounds from acid hydrolysates, strategies such as overliming, charcoal treatment, steam stripping, ion-exchange resin treatment, and biological methods such as enzymatic and microbial detoxification have been employed [1,16–18].

A number of cellulolytic microorganisms are known to produce cellulases and hemicellulases, which can convert cellulose and hemicellulose respectively into soluble monomeric or oligomeric sugars. These sugars further can be fermented into ethanol by a number of bacterial, yeast and filamentous fungi. The fermentation process would be economically viable only if both hexose and pentose sugars present in the hydrolysates are converted to ethanol. The ability to ferment pentoses is not widespread among microorganisms. The most promising yeast species identified so far, are *Candida shehatae*, *Pichia stipitis* and *Pachysolen tannophilus* [19–22]. But, the use of these yeasts for ethanol production from xylose at commercial level is limited mainly due to their slow fermentation rates, carefully regulated oxygen requirement, sensitivity to inhibitors and low ethanol tolerance [22]. Although significant improvements have been made in the microorganisms for the efficient fermentation of pentose sugars into ethanol, however, the bioconversion of pentoses to ethanol is still one of the major bottlenecks for ethanol commercialization effort. In this paper, an overview of bioconversion of pentose sugars into bioethanol using fermentation and molecular strategies for improvement of pentose-fermenting strains utilizing hemicellulosic sugars are discussed.

2. Hemicellulose

Among the different structural units of lignocellulosics, hemicellulose comprises almost 15–30% of the total dry weight and hence represents an important energy and material resource for bioconversion [6,23–25]. The hemicelluloses comprised both linear and branched hetero-polymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid (Fig. 1A). The xylose-rich hemicelluloses in both soft and hard wood are usually termed as xylans. The hemicellulose from hardwoods and agricultural residues are typically rich in xylan, while, softwood contains more mannan and less xylan [6,7,24,26].

2.1. Softwood hemicellulose

Galacto-mannans are the principal hemicelluloses in softwoods. Their backbone is a linear chain built up by 1,4-linked β-D-gluco-pyranose and β-D-manno-pyranose units (Fig. 1B). The mannose and glucose units in the backbone are partially substituted at C-2 and C-3 positions by acetyl groups, approximately 1 per 3–4 hexose units [6]. Arabino-glucuronoxylan is another major hemicellulosic sugar and is composed of 1,4-linked-β-D-xylopyranose units. This chain is substituted at C-2 by 4-o-methyl-α-D-glucuronic acid group with approximately two such units per ten xylose units. The xylose backbone is also substituted by α-L-arabino-pyranose units, on the average 1.3 residue per ten xylose units [6,25]. Arabino-galactan is a minor component in both softwoods and hardwoods. The backbone of this galactan is built up by 1,3-linked α-D-galacto-pyranose units, and almost every galactose unit is substituted at C-6 position.

2.2. Hardwood hemicellulose

The o-acetyl-4-o-methyl-glucurono-β-xylan (commonly known as glucuronoxylan) is the major component of hard wood hemicelluloses [6,27] (Fig. 1B). The xylan content varies between 15 and 30% in different hardwood species. The backbone of xylan consists of β-D-xylo-pyranose units linked by 1,4-bonds, while seven of ten xylose units are substituted by acetyl group at C-2 or C-3 position and in one of ten xylose units, the 4-o-methyl-α-D-glucuronic acid residue unit is linked at C-1, 2 positions to the hemicellulose backbone [6,25,28]. Gluco-mannan is another hemicellulose in hard woods (Fig. 1B), comprises 2–5% of the wood and is composed of β-D-gluco-pyranose and β-D-manno-pyranose units by 1,4-bonds. Depending on the wood species, the glucose:mannose ratio varies between 1:1 and 1:2 [6,24,25,29].

3. Pentose-fermenting microorganisms

During the last more than three decades, several laboratories round the world have examined the utilization of pentose sugars by different bacteria, fungi and yeasts for production of acids, alcohols and other fermentation products under cultivation conditions [30].

3.1. Bacteria

Most of the fungi cannot ferment pentoses anaerobically, while many bacteria can readily convert xylose to various products under anaerobic fermentation. The common pentose fermenting bacteria include *Bacillus macerans*, *Bacillus polymyxa*, *Klebsiella pneumoniae*, *Clostridium acetobutylicum*, *Aeromonas hydrophila*, *Aerobacter* sp., *Erwinia* sp., *Escherichia* sp., *Leuconostoc* sp., *Lactobacillus* sp., *Thermoanaerobacterium saccharolyticum*, and *Zymomonas mobilis* [22,31]. Among bacteria, thermophiles may be the best suited for the production of alcohol, polyols and ketones due to decreased cooling energy and low risk of contamination. Promising thermophilic pentose fermenting bacteria include *T. saccharolyticum*, *Clostridium thermohydrosulfurium*, *Clostridium thermosaccharolyticum*, *Clostridium thermosulfurogenes*, *Clostridium tetani* and *Thermoanaerobacter ethanolicus* [32–34]. These thermophiles have many industrially important properties such as wide substrate range, less biomass, no specific oxygen requirement, less risk of contamination and continuous recovery of volatile products. But, the low product tolerance and byproduct formation during the fermentation make these bacteria commercially unviable; however, high temperature fermentation may save cooling energy as compared to low temperature fermentation. Moreover, the genetic modification of thermophilic organisms would solve the problem of product tolerance.

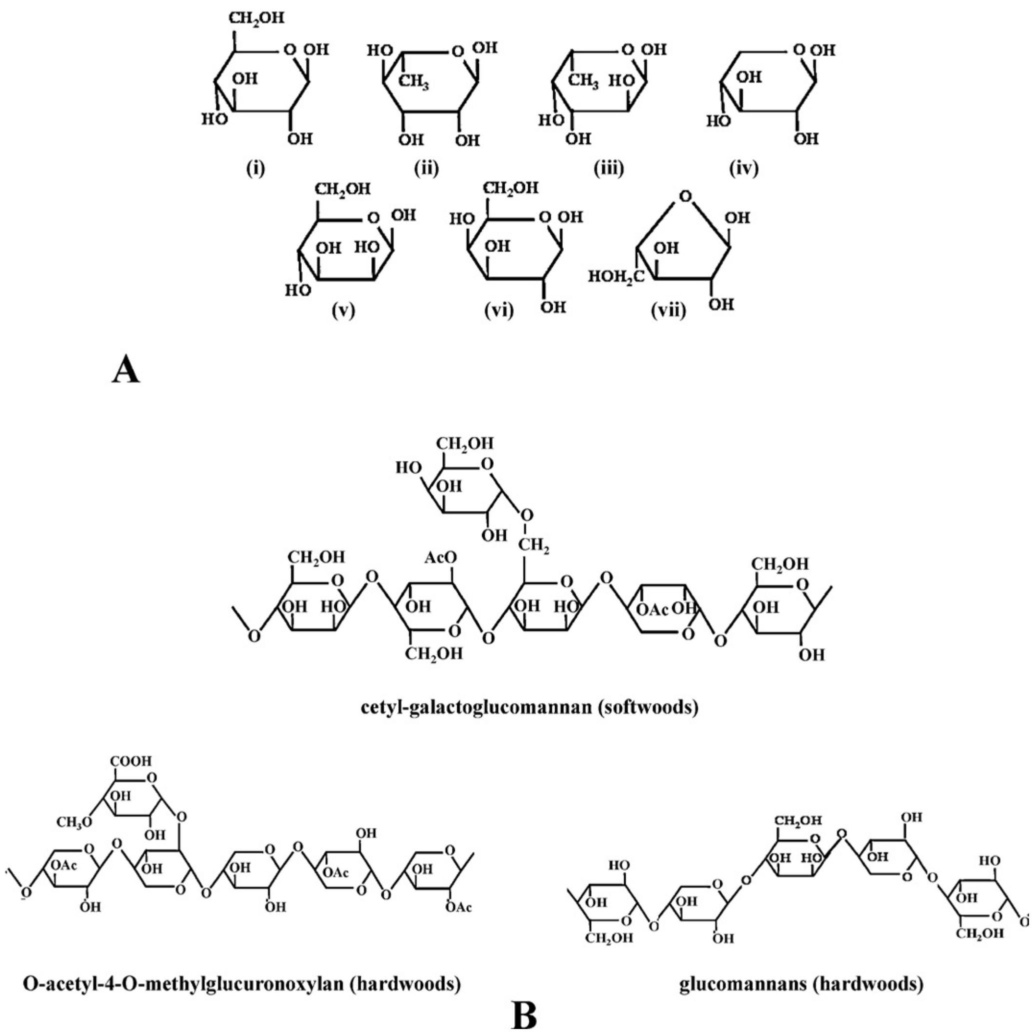


Fig. 1. (A) Structure of monosaccharides commonly present in xylan backbone: (i) β-D-glucopyranose; (ii) α-L-rhamnopyranose; (iii) α-L-fucopyranose; (iv) β-D-xylopyranose; (v) β-D-mannopyranose; (vi) β-D-galactopyranose and (vii) α-L-arabinofuranose; (B) structures of polymeric units of softwood and hardwood xylans.

3.2. Filamentous fungi

The filamentous fungi have been known to ferment sugars for more than 80 years. Several fungal species belonging to genera *Chalara* [35], *Fusarium* [36], *Rhizopus* [37], *Neurospora* [38], *Paecilomyces* [39] and *Trichoderma* [40] have potential for fermenting xylose. Some other useful fungal strains have also been studied that can ferment more complex natural cellulosics substrates as well. *Monilia* sp., *Neocallimastix* sp., *Trichoderma reesei* and *Fusarium oxysporum* have shown the ability for direct conversion of cellulose/hemicellulose to ethanol/acetic acid in single step fermentation [40,41]. Despite the pentose fermentation characteristics, these fungi have several physiological drawbacks such as, long fermentation period, low ethanol productivity, high viscosity fermentation broth, requirement of low critical oxygen levels and formation of byproducts in large amounts. However, a filamentous fungal system might be interesting because of their ability to grow on natural plant biomass, which yeast systems usually lack [22].

3.3. Yeast

The use of yeasts in conversion of carbohydrates to ethanol is known for generations. However, only a few strains are capable of converting pentoses [21]. The extensively studied yeast species for xylose fermentation are *P. tannophilus*, *C. shehatae*, *P. stipitis* and *Kluveromyces marxianus* [19,20,22]. Many other yeast species are also reported for their xylose-fermenting capabilities, which include *Brettanomyces*, *Clavispora*, *Schizosaccharomyces*, several other species of *Candida* viz., *C. tenius*, *C. tropicalis*, *C. utilis*, *C. blankii*, *C. friedrichii*, *C. solani* and *C. parapsilosis*, and species from *Debaromyces* viz., *D. nepalensis* and *D. polymorpha* [42,43]. Suh and coworkers have isolated a novel xylose-fermenting yeast '*Enteroramus dimorbhus*' from the microflora in the hindgut of beetles '*Odontotaenius disjunctus*' [44]. Our group has also screened 20 yeast strains for pentose fermentation recently, where only few strains of *Pichia* and *Pachysolen* showed pentose fermenting capabilities [45]. The pentose fermenting yeasts were observed to be less tolerant to pH, ethanol and hydrolysate inhibitors when compared to *Saccharomyces cerevisiae*. Moreover their inability to produce ethanol as major end-product from xylose is a major drawback for ethanol production [21,22,25].

4. Xylose metabolizing pathways

The initial metabolic pathway of D-xylose in microorganisms involves its conversion to D-xylulose followed by xylulose kinase

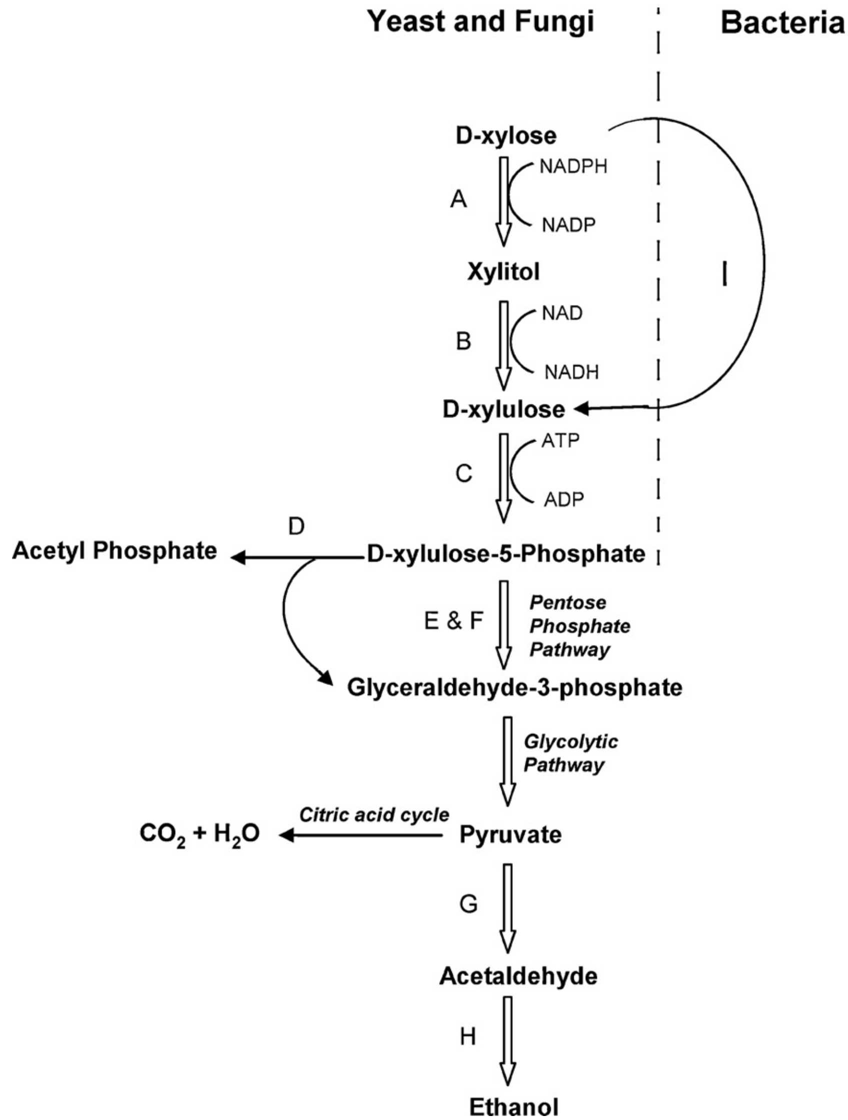


Fig. 2. Schematic diagram of D-xylose metabolism. (A) Xylose reductase; (B) xylitol dehydrogenase; (C) xylulokinase; (D) phosphoketolase; (E) transaldolase; (F) transketolase; (G) pyruvate decarboxylase; (H) alcohol dehydrogenase and (I) xylose isomerase.

reaction to D-xylulose-5-phosphate and then directed to pentose phosphate pathway (PPP). For D-xylose to D-xylulose conversion, bacteria generally use xylose isomerase (XI) enzyme, whereas yeasts and mycelial fungi employ a two step oxidation–reduction pathway [46]. In yeasts and fungi, the D-xylose is first reduced to xylitol by D-xylose reductase (XR) and subsequently oxidized to D-xylulose by xylitol dehydrogenase (XDH), which is further oxidized to form D-xylulose-5-phosphate. D-Xylulose is further metabolized via pentose phosphate pathway (PPP), in which the non-oxidative rearrangements of α-xylose-5-phosphate by ribulosephosphate-3-epimerase, transaldolase (TAL) and transketolase (TK) results in the formation of glyceraldehyde-3-phosphate and fructose-6-phosphate, which can be converted to ethanol by fermentative reactions of the Embden–Meyerhoff–Parnas (EMP) pathway [47] (Fig. 2).

Fermentative yeasts generally possess both aerobic and anaerobic pathways along with adaptive regulatory mechanisms. Even if they can metabolize D-xylose anoxically, D-xylose-fermenting yeasts require functional mitochondria and oxygen for growth, regardless of the carbon source. During typical aerobic xylose fermentation, ethanol concentration peaks sharply and then declines as consumption exceeds production. However, ethanol uptake is not observed in anaerobic xylose fermentation, where the TCA cycle is not operational [22,48].

5. Fermentation of pentose sugars

The general requirements of an organism for ethanol production from pentose sugar hydrolysate should be high ethanol yield, high productivity, good tolerance against inhibitors as well as high ethanol concentrations and ability to ferment at relatively low pH. *S. cerevisiae* is one of the most commonly used yeasts for ethanol fermentation using glucose. However, it does not have the ability of fermenting pentose sugars. The most promising yeast species identified so far for the pentose fermentation are, *C. shehatae*, *P. stipitis* and *P. tannophilus* [19,20]. Various studies have been carried out for the fermentation of xylose rich hydrolysates from different lignocellulosic materials (Table 1). Moniruzzaman achieved 78% theoretical ethanol yield during the fermentation of enzymatic hydrolysate of steam exploded rice straw, however, a 2–3 h lag due to diauxic phenomenal metabolic shift from glucose to

Table 1
Summary of research work on pentose fermentation using lignocellulosic biomass.

Substrates	Organism	Sugar (g/l)	Ethanol (g/l)	Ethanol yield (g/g)	Reference
Corn stover	*P. stipitis*	40	15.92	0.4	[97]
Prosopis juliflora	*P. stipitis*	18	7.1	0.39	[9]
Rice straw	*P. stipitis*	33	14.9	0.45	[155]
Sun flower seed hull	*P. stipitis*	34	11	0.32	[52]
Sugar cane bagasse	*Pachysolen tannophilus*	63.5	19	0.34	[156]
Sugar maple	*P. stipitis*	35	12.4	0.35	[51]
Corn stover	*P. stipitis*	40	15	0.37	[50]
Sugar cane bagasse	*Candida shehatae*	30	8.67	0.29	[1]
Corn stover	*P. stipitis*	60	25	0.42	[157]
Red oak wood chips	*P. stipitis*	36	14.5	0.4	[158]
Red oak spent sulfite liquor	*P. stipitis*	49	20.2	0.41	[159]
Wheat straw	*P. stipitis*	52	22.3	0.43	[160]
Corn cob	*Pichia stipitis*	30	10.4	0.34	[161]
Poplar	*P. stipitis*	39	12	0.31	[162]
Switch grass	*P. stipitis*	39	14	0.36	[162]
Rice straw	*Candida shehatae*	20	9	0.45	[19]
Rice straw	*P. stipitis*	15	6	0.4	[49]
L. camara	*P. stipitis*	16.8	5.16	0.33	[12]

xylose was also observed [49]. In another study using the acid and the auto-hydrolysate of rice straw, *C. shehatae* NCIM 3501 showed enhanced ethanol production in auto-hydrolysate (23.1 g/L) than in acid hydrolysate (20.0 g/L) because of lower inhibitor concentration [19]. The pentose fermentation process does not require intensive aerobic fermentation because of high cell mass synthesis, low ethanol yields and higher aeration energy consumption. However, aeration is required for the biomass production, which could be a major problem during the fermentation of non-detoxified hydrolysate. Interestingly, the fermentation of non-detoxified corn stover hydrolysate at higher aeration improved the ethanol production which was due to the higher xylose consumption translating higher biomass concentration [50]. In a similar study the degree of aeration showed a prominent effect on xylose utilization, ethanol production and xylitol minimization during the fermentation of membrane treated sugar maple hydrolysate using *P. stipitis* NRRL Y-7124 [51]. Further, different detoxification strategies were used by various researchers to enhance the ethanol production [1,52]. The removal of toxic inhibitors from fermentation broth significantly improved the ethanol yield (2.4-fold) and productivity (5.7-fold), compared to neutralized hydrolysate. Similarly, the fermentation of sugarcane bagasse acid hydrolysate with *C. shehatae* NCIM 3501 showed maximum ethanol yield (0.48 g/g) from ion exchange treated hydrolysate, followed by treatment with activated charcoal (0.42 g/g), laccase (0.37 g/g), overliming (0.30 g/g) and neutralization (0.22 g/g) [1]. While in another study, the sequential application of overliming with sodium sulfite addition was observed to be the best detoxification method for the sun flower seed hull acid hydrolysate, for maximum ethanol yield (0.32 g/g) and ethanol productivity (0.065 g/L/h) [52]. Recently, *P. stipitis* NCIM 3498 was used to ferment the detoxified acid hydrolysates of two lignocellulosic feedstocks (*Prosopis juliflora* and *Lantana camara*), resulting in an ethanol yield ranging from 60 to 70% of the theoretical yields [9,12].

Although significant work has been carried out on pentose fermentation, but no economically feasible process has been developed so far. Therefore, in order to achieve improved ethanol production, the major focus of research on pentose fermentation is shifting toward the exploration of improved fermentation strategies and strain improvement.

5.1. Pentose fermentation using whole cell immobilization

The immobilization technique offers many advantages such as cell recycling, economic extraction of the product, easy maintenance of specific growth and dilution rate, maintenance of high cell density, high productivity, good mixing and mass transfer with low risk of contamination [53–56]. Though the immobilized microorganisms showed enhanced volumetric productivities as compared to free microbes, however, in most of the cases, the xylose and glucose are not utilized at the same time due to catabolite repression [47]. In an attempt to ferment glucose and xylose simultaneously, Grootjen and coworkers used a co-culture cultivation of alginate immobilized *S. cerevisiae* and *P. stipitis* in a conventional bioreactor, where *P. stipitis* cells inoculum were taken in comparatively higher amount, which allowed more xylose utilization under anaerobic conditions and the fermentation appears to be simultaneous [53]. Furthermore, the modified stirred tank reactor (STR) system equipped with two teflon-made HPLC filters air diffusers with improved mixing and less shearing during co-culture strategy improved the ethanol yield up to 80% with calcium alginate immobilized *P. stipitis* and *S. cerevisiae* [54]. Interestingly, an agar sheet sandwiched between two chambered bioreactor has also been used for the co-immobilization of *S. cerevisiae* and *C. shehatae* during the mixed sugar (glucose and xylose) fermentation, however, the cell proliferation in the gel clogged the microporous membrane, which in turn limited the mass transfer [57]. In another study to overcome the problem of glucose catabolite repression, sieve plates adjusted STR with a movable device was used for the coculture of immobilized *Z. mobilis* and free *P. stipitis* to improve the fermentation efficiency [58]. Recently, a calcium alginate immobilized recombinant *S. cerevisiae* strain ZU-10 has been used for the fermentation of detoxified corn stover hemicellulosic hydrolysate, which showed the consumption of more than 92% xylose with an enhanced ethanol yield and productivities with higher tolerance to fermentation inhibitors [56].

Despite various improvements of these fermentations, the repeat culture with the same batch of immobilized microorganism under the same conditions resulted in decreased performance [58]. Alternatively yeast cells immobilized by self flocculation have shown many advantages such as no requirement of support matrix, maintained biomass and enhanced ethanol tolerance. Moreover flocculated yeast cells can be recovered by sedimentation from fermentation broth. However, CO_2 bubbles produced during ethanol fermentation can alter the settling zone and disturb the sedimentation of yeast floc, whereas a specially designed baffle can overcome this problem [59]. There are also some reports of yeast cells immobilization for biocatalysts development for simultaneous saccharification and fermentation (SSF). Fujita and coworkers constructed a yeast-based whole-cell biocatalyst displaying *T. reesei* xylanase II

on the cell-surface and showed xylan degradation by recombinant cells [60]. Significant attempts have also been made for the development of yeast cells displaying cellulase activities on the cell surface so as to decrease the usage of exogeneously added cellulase [61,62].

5.2. Pentose fermentation using recycling of cells

Increasing the cell density by cell recycling and cell retention has been a suitable way of increasing the volumetric productivity for slow growing microorganisms [63,64]. The cell recycling operation requires lesser amount of nutrients, achieves high dilution rate in continuous operations, decreases cell mass synthesis, and increases ethanol yields [57,65]. A high productivity system that involved a membrane bioreactor with cell recycling of *Z. mobilis* ZM4 capable of converting both glucose and xylose to ethanol had been developed [66]. Similar strategy was also applied during the continuous cultivation of a recombinant xylose fermenting *S. cerevisiae* TMB 3001 on a xylose-glucose mixture [64]. Interestingly, the recycled cells get adapted to the fermentation inhibitors present in the hydrolysate and showed better results in lignocellulose hydrolysate containing mixed sugars [63]. Pruwadi and coworkers found similar results, where continuous cultivation of high cell density flocculating yeast in toxic dilute acid hydrolysate of spruce residues in a single and serial bioreactor adapted to the medium and reduced the requirement of any detoxification [67]. Recently, a fuzzy optimization of continuous fermentation with cell recycling for ethanol production was carried out [68]. From the computational results, the overall productivity of the continuous fermentation process with cell recycling allowed a higher dilution rate with 7.3-fold higher productivity [68].

Though in most of the earlier reports, the cell enrichment was carried out by cell resettling, centrifugation, microfiltration and ultrafiltration, but recently hydroclone has been used to recover the cells of *S. cerevisiae* IROST 5209 from the fermentation broth [69]. The hydroclone system offers an advantage of negligible cell disruption [69]. Furthermore, implementation of cell recycling in industrial process is possible only if the effluent passes the membrane without fouling. Industrial effluents are not suitable for membrane filtration due to suspended particles, solid residues and high viscosity. However, decreasing the viscosity in industrial effluents by enzymatic treatment is one of the possible solutions of this problem [69,70].

5.3. Pentose fermentation using simultaneous saccharification and fermentation

Simultaneous saccharification and fermentation (SSF) approach has also been employed to decrease sugar inhibition to cellulase, achieve improved ethanol production, and to reduce the overall process cost since both the processes took place in single reactor. The SSF technique reduces the processing time, which in turn leads to increase the apparent volumetric productivity of ethanol [71]. The other advantages of this approach are shorter fermentation time and reduced risk of contamination with external microflora due to the high temperature of the process, the presence of ethanol in the reaction medium and the anaerobic conditions [30,71,72]. Interestingly, in a comparison between the separate hydrolysis and fermentation (SHF) and SSF of steam exploded corn stover, it was observed that SSF gave a 13% higher overall ethanol yield than SHF (72.4% versus 59.1% of the theoretical) [72]. However, the performance of the SSF process is limited by the competence between optimum temperatures for enzymatic saccharification and microbial fermentation [73]. Hence, the coexistence of two process conditions for low-temperature microbe and high-temperature fungal enzyme may hinder the efficacy of the SSF process.

To improve process efficiency, many microorganisms have been tested with cellulases produced by *T. reesei* mutants [30]. Besides, there are some microorganisms such as *Clostridium*, *Cellulomonas*, *Trichoderma*, with high cellulolytic and hemicellulolytic activity and are highly capable of fermenting mono- or oligo-saccharides into ethanol. Application of thermotolerant yeast strains may be beneficial in high temperature SSF processes, where fungal cellulase can also exhibit higher activity [73]. Ballesteros and colleagues tested different treatments to improve the thermotolerance of some species belonging to the genera *Saccharomyces*, and *Kluyveromyces* and the best results were obtained with *K. marxianus* LG [74]. The strain was further mutagenised and the mutated *K. marxianus* strain CECT 10875 achieved a SSF yield of 50–72% in 72–82 h using various lignocellulosic feedstocks such as *Populus* sp. and *Eucalyptus* sp., *Sorghum* sp. bagasse, wheat straw and *Brassica carinata* residue [75]. The efficacy of *Kluyveromces* was further confirmed by other researchers as well, where *Kluveromyces* strain NCIM 3358 in SSF experiments of softwood resulted in more than 70% ethanol conversion [76]. Similarly, during the SSF of lignocellulosic wastes with this thermotolerant yeast at 10% (w/v) initial substrate consistency, a final ethanol concentration of 2–2.5% was obtained after 72 h [77].

In another strain improvement report, a thermophilic anaerobic bacterium *T. saccharolyticum*, capable of utilizing xylan and biomass derived sugars, was engineered by removing the genes encoding acetate kinase, phosphate acetyl transferase and D-lactate dehydrogenase. The engineered thermophilic strain ALK2 thus obtained was able to reduce the requirement of externally added fungal cellulase by 2.5-fold [34]. Recently, a respiratory-deficient mutant of the thermotolerant yeast *Candida glabrata* (Cgrd1), was subjected to ethanol production by high-temperature SSF under aerobic conditions to achieve maximum ethanol (17.0 g/L) within 48 h at 66.6% of its theoretical yield and with 0.35 g/L h productivity [78].

However, more efforts are required in the development of microorganisms for economically feasible industrial ethanol production from pentose sugars. Since enzymatic hydrolysis is the rate-limiting step in SSF, increasing the rate of hydrolysis will lower the cost of ethanol production via SSF [30].

6. Strain improvement for pentose fermentation

6.1. Strain improvement through mutagenesis

There are several reports where mutagenised recombinant strains showed enhanced ethanol production over their parent strains [4,79–81]. In an early report, a recombinant *S. cerevisiae* strain TJ1 mutagenised with ethyl methane sulfonate (EMS) was found to have lower XR activity but high XD and xylulokinase (XKS) activities than the parent strain, which in turn resulted in 1.6-fold increase in ethanol production [82]. Further, a mutant of *S. cerevisiae* TMB 3001 capable of utilizing xylose under anaerobic condition was developed by sequential EMS mutagenesis and adaptation of the mutant strain under microaerobic and anaerobic conditions [83]. Later on similar strategy was used to develop two EMS mutagenised *S. cerevisiae* strains 3399 and 3400 with improved growth on xylose [80]. Since the most efficient xylose utilizing microbes are not able to metabolize the pentose sugars anaerobically, the strategies of natural selection and random mutations were also tested [84]. Besides EMS, several other mutagens had also been used to obtain mutants derepressed for pentose metabolism. Sreenath and Jeffries used 2-deoxyglucose (2-DOG) mutated strain, showing considerable improvement in xylose utilization [63]. While in another report, a UV mutagenised *P. stipitis* NRRL Y-7124 strain was found to produce higher ethanol than the wild strain [85].

Site-directed mutagenesis is another strategy used to obtain the mutants for better xylose fermentation. Watanabe and coworkers used multiple site-directed mutagenesis of the NAD^+-dependent XDH from *P. stipitis* and introduce a structural zinc atom for the complete reversal of the coenzyme specificity [81]. The selected mutants were found to exhibit significant thermostability and enhanced catalytic activity with $NADP^+$. Similarly, several PsXDH mutants were generated with complete reversal of coenzyme specificity toward $NADP^+$ by multiple site-directed mutagenesis within the coenzyme-binding domain and with increased thermostability by refining the structural zinc-binding loop without affecting their activities [86]. In addition one of the *S. cerevisiae* mutant (MA-R5) under the control of a strong constitutive promoter showed particularly high ethanol production from xylose and low xylitol yield by fermentation of not only xylose as the sole carbon source, but also a mixture of glucose and xylose [87–89]. Additionally using this approach, an ethanologenic *Escherichia coli* mutant, devoid of foreign genes, has also been developed by combining the activities of pyruvate dehydrogenase and the fermentative alcohol dehydrogenase and the mutant was found able to ferment glucose or xylose to ethanol with 82% ethanol yield under anaerobic conditions [79].

6.2. Strain improvement through protoplast fusion

Protoplast fusion provides characteristic advantage such as promotion of high frequencies of genetic information between organisms for which poor or no genetic exchange has been demonstrated or which are genetically uncharacterized [90–92]. In the presence of a fusogenic agent such as polyethylene glycol (PEG), protoplasts are induced to fuse and form transient hybrids or diploids. Several reports on protoplast fusion between pentose and hexose utilizing yeasts showed efficient utilization of both sugars with higher biomass yield. Heluane and coworkers successfully transferred the genes of xylose utilization from *P. tannophilus* to *S. cerevisiae* [90]. The hybrids reassembled the *S. cerevisiae* parent morphologically but displayed the ability to use the pentose sugars (xylose) similar to *P. tannophilus*. The same has been supported by other workers, where a fusant of *Schizosaccharomyces pombe* and *Lentinula edodes* were found to utilize xylan as carbon source [91]. In an another study, the protoplasts of thermotolerant *S. cerevisiae* and mesophilic xylose-utilizing *C. shehatae* were fused by electrofusion and the fusant yeast gave an ethanol yield of approximately 0.459 g/g with productivity of 0.67 g/L/h and fermentation efficiency of 90% and showed higher temperature tolerance up to 40 °C as well [92]. Moreover, using a combinatorial approach, a xylose fermenting fusant (F6) of *C. shehatae* and *S. cerevisiae* was developed, showing improved ethanol production (28%) than its parental strain [93]. In this strategy the *C. shehatae* was first adapted for ethanol tolerance and then mutagenised by UV irradiation and thus a respiration deficient mutant RD-5 was selected. Further, the protoplasts of RD-5 and *S. cerevisiae* were fused and the resultant fusant strain F6 showed 28% higher ethanol production than the parent *C. shehatae* stain, with the production level of 18.75 g/L from 50 g/L xylose. Recently a strategy of genome shuffling was also used, in which the genomes of 6 UV mutagenised *P. stipitis* strain (WT, PS302, GS301, GS302, GS401 and GS402) were shuffled and after the 3rd and 4th rounds of genome shuffling, putative improved mutant colonies were pooled, re-grown and spread on hardwood spent sulphite liquor (HWSSL) gradient plate again [94]. *P. stipitis* WT and PS 302 could not grow in any of the HWSSL concentrations, while 2 mutants (GS401 and GS402) from the 4th round could grow in 80% (v/v) HWSSL while another 2 mutants (GS301 and GS302) from the 3rd round could grow in 85% (v/v) HWSSL. Thus the study concluded that the mutated strains showed improved inhibitors tolerance against HWSSL [94].

6.3. Strain improvement through adaptation

Fermentation of wood-derived hydrolysates is sometimes problematic because of the toxic inhibitors released during thermochemical hydrolysis. However, the adaptation approach can be an alternative means to improve the microbial strains [95–97]. There are several reports on enhancement of ethanol yield and productivity using adapted strains of *P. stipitis* and *C. shehatae* for the fermentation of undetoxified or partially detoxified hydrolysates [97–99]. For instance, an ethanologenic yeast when adapted against inhibitors by repeated sub-culturing in a medium with furfural and HMF up to a concentration of 10–20 mM was found to grow more efficiently than its parent strain in the presence of inhibitors [98]. Another strategy of natural selection and breeding was used to develop non-recombinant strains of *S. cerevisiae* that could grow efficiently on xylose [99]. By breeding and natural selection over 23 mating cycles and 1463 selection days, a non-genetically modified *S. cerevisiae* (MBG-2303) was obtained, which grew aerobically on xylose and demonstrated 57-fold higher biomass production than the control strain [99]. Later on, it was demonstrated that adaptation of *P. stipitis* CBS 6054 in solid agar produce more ethanol (19.4 g/L) than liquid adapted (18.4 g/L) and unadapted strains (16.3 g/L) [95]. Recently, studies were carried out on adaptation of *P. stipitis* CBS 5776 strain which on fermentation of steam exploded prehydrolysate of corn stover showed improved ethanol yield of 15.92 g/L with 80.34% theoretical yield [97].

Moreover, the evolutionary adaptation approaches have also been applied to recombinant strains to improve their fermentation capability. Lawford and group improved the xylose-fermenting recombinant strain *Z. mobilis* 39767 to tolerate higher concentration of acetic acid by subculturing in a medium containing 10–50% of hydrolysate and the adapted isolates demonstrated a significant improvement in ethanol productivity compared to un-adapted strains [100]. Similarly, an engineered *E. coli* KO11 was developed to tolerate high ethanol concentration using a long term adaptation strategy of alternative serial selections for liquid and solid medium. The mutants thus developed, i.e., LY01, LY02 and LY03 demonstrated more than 50% survival rate in 10% ethanol (0.5 min exposure) and also reduced the fermentation time [96]. In almost all previous efforts of evolutionary adaptation the organism was first subjected to genetic engineering, which was followed by adaptive selection [83,101,102]. However, recently a new strategy consisting genetic engineering, mutation with EMS followed by two-step evolutionary adaptation (under sequential aerobic and oxygen limited conditions) has also been attempted [4]. The strain thus developed showed fourfold increase in its specific growth rate compared to the parental strain. Interestingly the activity of critical enzymes of xylose metabolism (XR, XDH and XK) remain unchanged suggesting that chemical mutagenesis and evolutionary adaptation might have created a new genetic traits making the mutants capable of xylose metabolism [4].

6.4. Strain improvement through genetic manipulation

Substantial progress in the genetic engineering of different microbes for the conversion of xylose or pentose sugars to ethanol has been achieved [4,88,103]. Although the genetically engineered host strains of bacteria and yeast showed tremendous improvement in final ethanol yields and efficient utilization of pentose sugars (Table 2), the information about the usage of genetically modified organisms for large scale pentose fermentation is scarcely available [25].

6.4.1. Genetic engineering of E. coli

Ingram's group have done extensive work on the development of efficient recombinant *E. coli* strains for ethanol production.

Table 2
List of pentose utilizing recombinant yeasts and bacterial strains.

Strain	Sugar/sugar mix* used (g/l)	Ethanol production (g/l)	Ethanol yield (g/g)	Ethanol productivity (g/l/h)	Reference
E. coli KO11	80 X	41.6	102	0.87	[107]
E. coli KO11	90 X	41	89	0.85	[96]
E. coli KO11	140 X	59.5			[96]
E. coli LY01	140 X	63.2	88	0.66	[96]
E. coli FBR5	95 X	41.5	90	0.59	[110]
E. coli FBR5	A:X:G 15:30:30	34.0	90	0.92	[110]
E. coli SE2378	60 X	27.8	82	NA	[79]
T. saccharolyticum ALK-2	70X	33.1	92	2.2	[41]
Z. mobilis CP4	25 X	11.0	86	0.57	[115]
Z. mobilis CP4	G:X 65:65	24.2	95	0.81	[115]
Z. mobilis ATCC 39767	G:X:A 30:30:20	33.5	82-84	0.82-0.65	[117]
Z. mobilis CP4	60 X	23	94	0.32	[163]
Z. mobilis ZM4	G:X 65:65	62	90	1.29	[66]
Z. mobilis AX101	A:G:X 20:40:40	42	84	0.61	[119]
S. cerevisiae PRD1	21.7 X	1.6	14	0.07	[164]
S. cerevisiae TJ1	50 X	2.7	10.6	0.02	[165]
S. cerevisiae 1400	G:X:A:Gal 31:15:10:2	22	90	0.92	[166]
S. cerevisiae 1400	80 X	27	66	1.12	[166]
S. cerevisiae 1400	50 X	1.5	6		[121]
S. cerevisiae H158	80 X	21.6	54	NA	[123]
S. cerevisiae TMB 3008	50 X	19	76	0.27	[48]
S. cerevisiae TMB 3251	50 X	17	68	0.24	[48]
S. cerevisiae TMB 3255	50 X	20.5	82	0.29	[48]
S. cerevisiae H 2684	50 X	20.5	82	NA	[131]
S. cerevisiae TMB 3400	20 X	5	50	NA	[80]
S. cerevisiae TMB 3001	10 X	2.4	48	NA	[83]
S. cerevisiae BH42	G:X 50:50	28	56	NA	[167]
S. cerevisiae TMB 3120	10 X	4.6	92	0.064	[168]
S. cerevisiae TMB 3050	50 X	14.5	58	NA	[103]
S. cerevisiae RWB 217	20 X	8.6	86	NA	[101]
S. cerevisiae TMB 3270	50 X	18	72	0.32	[135]
S. cerevisiae TMB 3400	50 X	17	68	0.12	[128]
S. cerevisiae L2162	40 X	5.2	0.13	0.032	[130]
S. cerevisiae DR PHO13	40 X	10	0.25	0.093	[130]
S. cerevisiae TMB 3066	50 X	21.5	86	0.073	[128]
S. cerevisiae CMB JHV	20 X	6.4	0.32	NA	[169]
S. cerevisiae BP10001	20 X	6.8	0.34	NA	[139]
S. cerevisiae MA-N5	45 X	15.8	0.36	0.24	[88]
S. cerevisiae RBW 202-AFX	50 X	19.5	78	NA	[170]
S. cerevisiae INVSC1	50 X	19.5	0.39	0.043	[171]
S. cerevisiae ADAP8	20 X	8.6	0.43	0.07	[172]
S. cerevisiae MA-R4	45 X	15.75	0.35	0.36	[84]
S. cerevisiae MA-R5	45 X	16.65	0.37	0.50	[84]
S. cerevisiae ZU-10	80 X	30.2	75.6	0.50	[56]
S. cerevisiae LEK 513	50 X	8.13	32.5	0.113	[4]

* A, arabinose; G, glucose; Gal, galactose; M, maltose; and X, xylose.

They eliminated the dependence of host on alcohol dehydrogenase (ADH) activity by combining *adh* B and *pdc* (coding for pyruvate decarboxylase, PDC) genes of *Z. mobilis* to form *pet* operon [104,105]. Considering a number of factors influencing ethanol production such as substrate range and growth conditions, *E. coli* strain ATCC 11303 was chosen as the host for the pet plasmid [106]. Further, to improve the genetic stability, the *pet* operon was integrated into the chromosome of ATCC11303 [99]. Since the strain containing the integrated genes produced only low levels of ethanol, the spontaneous mutation strategy generated a hyper ethanol producing KO4 strain. This strain was further modified by deletion of the succinate production gene (*frd*) to prevent the formation of succinate, a major byproduct of *E. coli* metabolism. The finally developed strain KO11 was able to convert glucose and xylose to ethanol at theoretical yields of 100% in rich media containing ample yeast extract [107]. To further improve pentose fermentation by KO11, a number of spontaneous mutants defective in glucose transport were selected and two such strains SL28 and SL40 when fermented using individual or mixture of xylose and glucose produced ethanol more efficiently (by 20%) than the parent strain KO11 [108]. The recombinant *E. coli* strain was further improved to achieve better xylose fermentation, the glycolytic flux and the growth rate of recombinant strain [109]. However, from an industrial point of view, these recombinant strains still had the drawback of requiring nutrient rich medium for ethanol production. In order to overcome, a lactate producing recombinant of KO11 was reengineered for

ethanol production by deleting genes encoding for fermentative routes for NADH and randomly inserting a promoter-less cassette containing the complete *Z. mobilis* ethanol pathway into KO11 chromosome [96].

Using different approach, Dien and coworkers developed new ethanologenic strains of *E. coli* such as FBR3, FBR4 and FBR5 [110]. These strains were generated by transforming a xylose-utilizing isolate of strain *E. coli* FMJ39 with plasmid pLOI297 having *Z. mobilis* pyruvate to ethanol converting enzymes. Alternatively a homo-ethanologenic strain of *E. coli* SE2738 from wild type *E. coli* K-12 W3110 was also developed, where the mutant strain exhibited 82% theoretical ethanol yield when grown on xylose under anaerobic conditions [79]. In a recent study, *E. coli* cells for efficient ethanol production from hexoses and pentoses were developed using elementary mode analysis to dissect the metabolic network into its basic building blocks [111]. During this strategy the functional space of the central metabolic network was reduced, with eight gene knockout mutations, from over 15,000 pathway possibilities to 6 pathway options that support cell function. Furthermore the elimination of three additional genes resulted in a strain that selectively grows only on pentoses, even in the presence of glucose, with a high ethanol yield. Later on using the similar approach, a glycerol to ethanol converting *E. coli* strain was designed by reducing the functional space of central carbon metabolism to a total of 28 glycerol utilizing pathways [112]. More recently an attempt has been made to engineer *E. coli* for the production of ethanol from fatty acid feedstocks, resulting in ethanol yield higher than the theoretical maximum obtained from sugars [113].

6.4.2. Genetic engineering of Z. mobilis

The xylose utilization in *Z. mobilis* was developed by integrating XI and XKS from *E. coli*, *Xanthomonas campestris* and *K. pneumoniae* in its genome [114,115]. Using the similar approach, Zhang and coworkers developed recombinant *Z. mobilis* CP4 showing activity for all four enzymes (XI, XKS, tal and tkt), which when grew on xylose as the sole carbon source resulted in 86% theoretical ethanol yield [115]. In another work, the constructed operons encoding xylose assimilation and pentose phosphate pathway enzymes were transformed into *Z. mobilis* to generate pZB5 strain for the effective fermentation of xylose to ethanol [36,115]. Further to construct improved strains with higher ethanol productivities and yields, pZB5 was transformed into *Z. mobilis* ethanol producing strain ZM4; ATCC 31821, which showed the capability of converting a mixture of 65 g/L of glucose and 65 g/L of xylose to 62 g/L ethanol in 48 h with an overall yield of 0.46 g/g [66]. Following similar approach, another group incorporated five genes of arabinose utilization from *E. coli ara A* (coding for L-arabinose isomerase), *ara B* (coding for L-ribulokinase), *ara D* (coding for L-ribulose-5 phosphate-4-epimerase), *tal* and *tkt* in *Z. mobilis* ATCC 39767 [116]. The resultant strain showed more than 90% ethanol yield from L-arabinose. However, 40% of the cells lost their ability to ferment arabinose when grown on complex medium. A number of other improvements have also been made in Z. *mobilis* strains and the new strain *Z. mobilis* AX101 fermented both arabinose, xylose and glucose and carried seven necessary recombinant genes as part of chromosomal DNA [117–119]. The co-fermentation process yield from *Z. mobilis* AX101 was about 84%, with preferential order in sugar utilization as glucose followed by xylose and arabinose [119]. However, these strains showed acetic acid sensitivity [119]. To address the problem of sensitivity to toxic fermentation inhibitors, a new strain of *Z. mobilis* ZM4/AcR (pZB5) was developed with increased acetate resistance that has enhanced performance in the presence of 12 g sodium acetate per litre at pH 5 [61]. *Z. mobilis* ZM4 produced near theoretical yields of ethanol with high specific productivity and was able to ferment both C-5 and C-6 sugars. The transformed *Z. mobilis* ZM4 performed best under anaerobic conditions, but also exhibited tolerance to aerobic conditions. However, the genetic and physiological basis of ZM4's response to various stresses has only been understood poorly. Recently, transcriptomic and metabolomic profiles for ZM4 under aerobic and anaerobic fermentations have been elucidated using microarray, high-performance liquid chromatography and gas chromatography–mass spectrometry (GC–MS) analysis [120].

6.4.3. Genetic engineering of S. cerevisiae

S. cerevisiae produces ethanol from hexose sugars but cannot ferment xylose or arabinose. However, the yeast is able to metabolize a xylose isomer, xylulose and recombinant DNA technologists have taken advantage of this in creating xylose-fermenting strains. Ho and coworkers were the first to successfully create a recombinant *S. cerevisiae* strain capable of effective xylose fermentation and xylose and glucose co-fermentation [121]. The recombinant plasmids with XR and XDH genes from *P. stipitis* and XKS gene from *S. cerevisiae* were transformed into *S. cerevisiae* for the co-fermentation of glucose and xylose. In contrasting report the overexpression of endogenous XKS from *S. cerevisiae* was found to inhibit its growth on xylulose [122]. Similarly some other workers have also reported about lower consumption of xylose in such strains [123–125]. However, Toivari and coworkers reported successful xylose fermentation to ethanol through over-expression of the endogenous XKS 1 and PsXR and XDH genes [126]. Recently following similar strategies, the improved xylose utilization and high ethanol production have been reported by various groups [88,127–129]. Based on these reports it can be concluded that the controlled overexpression of XKS gene in *S. cerevisiae* improved the xylose consumption as well as ethanol production. Jeffries and coworkers identified some interesting spontaneous or chemically induced mutants of recombinant *S. cerevisiae* that can overcome the growth inhibition caused by overexpression of ScXKS and PsXKS gene [130].

In order to achieve reduction in xylitol formation during xylose fermentation, recombinant *S. cerevisiae* strains expressing PsXR and PsXDH and overexpression of ScXKS were constructed that lowered the oxidative PPP activity through the GND1 (6-phosphogluconate dehydrogenase) and ZWF1 genes (glucose-6-phosphate dehydrogenase) [48]. These mutants showed increase in ethanol yield and xylose consumption rate compared to the parent strain. Furthermore an attempt to overexpress *Kluyveromyces lactis* GDP1 gene (NADP-dependent glyceraldehyde 3-phosphate dehydrogenase, GAPDH) in a xylose-fermenting *S. cerevisiae* strain was also found to enhance ethanol production [131].

In another approach to enhance ethanol yields, the metabolic flux toward ethanol formation appeared to be a significant strategy to improve the intracellular cofactor concentrations in *S. cerevisiae* [132]. The impact of over-expression of NADH kinase (encoded by the POS5 gene) on glucose and xylose metabolism in recombinant xylose-utilizing *S. cerevisiae* has also been studied [133]. The expression of NADH kinase in cytosol instead of mitochondria redirected the carbon flow from CO_2 to ethanol during aerobic growth on glucose, whereas under anaerobic growth the flux directed toward ethanol and acetate fermentation. The cytosolic NADH kinase appeared to revert these effects during anaerobic metabolism of xylose by channeling carbon flow from ethanol to xylitol [133]. The heterologous expression of a xylose isomerase (XI) may also be a good approach to enable yeast cells to metabolize xylose. In this aspect, Brat and coworkers screened nucleic acid databases for sequences encoding putative XIs and cloned them to express a highly active XI from the anaerobic bacterium *Clostridium phytofermentans* in *S. cerevisiae*, which resulted in an efficient metabolism of D-xylose as the sole carbon and energy source by recombinant yeast cells [134].

Alternative protein engineering approach has also been investigated to reduce xylitol formation and enhancing ethanol yield

using recombinant *S. cerevisiae*. Using this approach an improved ethanol production accompanied by decreased xylitol formation was achieved in recombinant *S. cerevisiae* expressing mutated PsXR (having reduced affinity for NADPH), PsXDH and ScXKS [135]. In this aspect, several NADH-preferring XR mutants from *Candida tenuis* have been developed [136–139]. Recently one such *S. cerevisiae* strain harboring the K274R-N276D CtXR double mutant showed enhanced ethanol production with decreased xylitol formation [140]. The heterologous expression of xylose specific transporters in recombinant *S. cerevisiae* for improved ethanol production has also been tested. The SUT1 gene [141] coding a sugar transporter in *P. stipitis*, has been successfully expressed in *S. cerevisiae* [142]. Moreover, the glucose/xylose-facilitated diffusion transporter and glucose/xylose symporter from *Candida intermedia*, encoded by Gxf1 and Gxf2 genes [143] have been expressed in *S. cerevisiae* [144], where the recombinant xylose-fermenting *S. cerevisiae* strain harboring Gxf1 showed faster xylose uptake and ethanol production [145]. Recently, a combinatorial approach of genetic engineering, chemical mutagenesis and evolutionary adaptation has been used to improve the xylose utilization. The *S. cerevisiae* strain W303-La was introduced with XI and XKS gene from *P. stipitis* NRRL7124 to make *S. cerevisiae* LEK 122. Thereafter, the selected strain was chemically mutagenised with EMS followed by their evolutionary adaptation for xylose utilization and growth under oxygen-limited conditions [4].

7. Future prospects

Ethanol has always been considered a better alternate to gasoline, as it reduces the dependence on fossil fuel reserves and promises cleaner combustion leading to a healthier environment. Interestingly, the world's focus is switching over from corn and sugarcane to second generation feedstocks as renewable raw material for production of bioethanol [146–149]. In recent years, significant developments in hemicellulose to ethanol conversion have been achieved. However the industrial activities for bioethanol production are limited mainly because of the cost of raw material (lignocellulosic biomass) processing to obtain high yield of fermentable sugars and unavailability of efficient fermentation strategies. The process of hemicellulose conversion to bioethanol requires adequate breakdown of lignocellulosic biomass with maximum pentose sugar yield and efficient utilization of pentose sugars during fermentation. Although various pretreatment methods and their effects on biomass composition and sugar yields have widely studied and described in literature but efficient fermentation of high yield hemicellulosic hydrolysates is essential to maximize the ethanol productivity and subsequent cost effective ethanol recovery [25]. The generation of microbial inhibitors during the pretreatment process is a major concern, which affects the economics of bioethanol production from lignocellulosic materials. Different detoxification strategies have been applied to remove these inhibitors from hemicellulosic hydrolysates to improve their fermentability [9,12,21,150], this additional step increases the overall ethanol production cost. There is an imperative need for bioprospecting of new robust microbial strains capable of converting the pentose sugars efficiently from un-or partially detoxified hydrolysates [151].

Since the efficient utilization of pentose sugars is a prerequisite for cost effective and sustainable ethanol production, the major research has now focused on further improving ethanol productivities using genetic modification and chemical additives [152,153]. Although several research groups have taken lead in developing a number of improved xylose utilizing recombinant organisms, it appears that xylose utilization and fermentation capabilities are commercially unattractive. Therefore, the genetic engineering approaches should be more focused on developing new improved strains with higher substrate tolerance and improved production kinetics. Additionally, the better elucidation of pentose sugars transport at the molecular level and characterization of kinetic and regulatory properties, including quorum-sensing mechanisms, should be given high priority because it may provide the basis for developing improved strains that can simultaneously utilize pentose and hexose sugars released during biomass hydrolysis. High-throughput screening techniques and better expression systems for efficient production of membrane proteins, and enzyme complexes such as cellulosomes are in progress.

Recently, the chromosomal integration of genes encoding the xylose utilization pathway enzymes into industrially applied yeast strains are reported necessary for large scale fermentation of xylose from lignocellulosic biomass to ethanol [89]. Therefore, further studies at micro levels are also required to combine the functional genomics with metabolic engineering strategies, which may provide further clues for developing robust strains. Moreover, the approach of flux analysis to divert or increase the activity of certain crucial enzymes in ethanol production with efficient xylose utilization needs more attention.

To make the bioethanol production process successful at industrial scale with reduction in capital and operation cost, some integrated unit operations using robust microorganisms for better product yields should be adopted [154]. An ideal up-scaling strategy needs to be fully integrated to evaluate the complete system (e.g., enzymes, nutrients, product yields and titers, and fermenting yeasts) with sufficient flexibility to investigate alternative process configurations. From a process scale-up perspective, the challenges lie not only with finding the most efficient organism for hemicellulose conversion but also to make an intelligent use of the entire process integration, a biorefinery concept.

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